



US006080553A

United States Patent [19][11] **Patent Number:** **6,080,553****Sogabe et al.**[45] **Date of Patent:** ***Jun. 27, 2000**[54] **CREATINE AMIDINOHYDROLASE,
PRODUCTION THEREOF AND USE
THEREOF**3,907,644 9/1975 Mollering et al. 195/99
5,451,520 9/1995 Furukawa et al. 435/227[75] **Inventors:** **Atsushi Sogabe; Takashi Hattori;
Yoshiaki Nishiy; Yoshihisa
Kawamura, all of Tsuruga, Japan****FOREIGN PATENT DOCUMENTS**62 091182 4/1987 Japan .
07 265074 10/1995 Japan .[73] **Assignee:** **Toyo Boseki Kabushiki Kaisha,
Osaka, Japan***Primary Examiner*—Elizabeth Slobodiansky
Attorney, Agent, or Firm—Leydig, Voit & Mayer, Ltd.[*] **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).**ABSTRACT**

[57] A creatine amidinohydrolase having the following physico-chemical properties:

Action: catalyzing the following reaction;
creatine+H₂O→sarcosine+urea**Optimum temperature:** about 40–50° C.**Optimum pH:** pH about 8.0–9.0**Heat stability:** not more than about 50° C. (pH 7.5, 30 min)**Km value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:** about 3.5–10.0 mM**Molecular weight:** about 43,000 (SDS-PAGE)**Isoelectric point:** about 3.5,

a method for producing said enzyme, comprising culture of microorganism producing said enzyme, a method for the determination of creatine or creatinine in a sample using said enzyme, and a reagent therefor.

[21] **Appl. No.:** **08/799,897**[22] **Filed:** **Feb. 13, 1997****Foreign Application Priority Data**

Feb. 13, 1996 [JP] Japan 8-025435

[51] **Int. Cl.⁷** **C12Q 1/34; C12N 9/78;
C12N 1/20; C12N 1/00**[52] **U.S. CL.** **435/18; 435/227; 435/192;
435/252.3; 435/320.1; 435/829; 435/252.33**[58] **Field of Search** **435/18, 227, 252.1,
435/320.1, 829, 192, 252.3, 252.33****References Cited****U.S. PATENT DOCUMENTS**

3,806,420 4/1974 Holz et al. 195/66

23 Claims, 2 Drawing Sheets